
UNIVERSITI PUTRA MALAYSIA

# STRUCTURAL, OPTICAL AND DIELECTRIC PROPERTIESOF LITHIUM FLUORO AND CHLOROAND MAGNESIUM CHLORO PHOSPHATE GLASSES

LOH YEN NEE

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# PREVALENCE OF GENETIC DIVERSITY BETWEEN GROUP A AND GROUP B OF RESPIRATORY SYNCYTIAL VIRUS ISOLATED FROM HOSPITALIZED PATIENTS IN A TEACHING HOSPITAL IN KUALA LUMPUR.

**By**

**VINOMARLINI A/P GUNASAGARAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science**

**August 2005**

**DEDICATION**

*To my mom and dad*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

**PREVALENCE OF GENETIC DIVERSITY BETWEEN GROUP A AND GROUP B OF RESPIRATORY SYNCYTIAL VIRUS ISOLATED FROM HOSPITALIZED PATIENTS IN A TEACHING HOSPITAL IN KUALA LUMPUR.**

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**August 2005**

**Chairman : Zamberi Sekawi, M.D.**

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Respiratory Syncytial virus (RSV) is one of the most important causes of lower viral respiratory tract infection resulting in hospital admission among infants and early childhood worldwide. The virus is a seasonal virus, with annual outbreaks occurring during the winter in temperate climates and during the rainy season in tropical climates. The main objective of this study was to determine the prevalence of RSV group A and B in the Klang Valley area by using Polymerase Chain Reaction (PCR) as a rapid and simultaneous detection of RSV. In this study, Polymerase Chain Reaction (PCR) method was used to detect the RSV and seminested PCR was used to subtype RSV into groups. This method is more sensitive and reliable compared to the current method used for detecting RSV which is by using direct immunofluorescence immunoassay. The

detection and subtyping of RSV both used the amplification of F and the G genes of RSV. The primer from the F gene region was used as the antisense primer for both detecting and subtyping while primer from different parts of the G gene region were used as the sense primer for detecting and subtyping of RSV respectively.

Random Amplified Polymorphic DNA (RAPD) technique was performed in this research to study the diversity of twenty RSV isolates. Four out of fifteen primers that were screened for reproducible band yielded clear multiple bands. According to the dendrogram generated from the RAPDistance software program, RSV isolates were distinctly separated into their own groups based on the year of isolation. The percentage of similarity among these isolates ranged from 33% to 95% while the Nei and Li's genetic distance obtained ranged from 0.0333 to 0.471.

The data obtained from this study only covers the Klang Valley area as all of the samples were collected from patients that were admitted in the pediatric ward of University Malaya Medical Center, Kuala Lumpur. All the samples were obtained from September 2002 until March 2004. The samples were collected from patients age ranging from one month to one year. Out of the twenty RSV samples obtained, thirteen of the RSV isolates were from the male patients and seven were from the female patients. The highest rate of infection occurred in the Malay community followed by the Chinese and the Indian. The chi-square test was done in order to determine whether the clinical data such as age, gender and the ethnicity was significant with the RSV infection. This study shows that,

the age, gender and the ethnicity of the patients were not significant and therefore, no relationship could be observed between the demographic data and the RSV infection.

From this study, RSV was successfully detected in 20 samples by PCR method. The F and G genes were amplified to detect and subtype RSV into groups A and B. The incidence of RSV A was much higher from September 2002 until March 2004 compared to the RSV B. Therefore, RSV A is prevalent in the Klang Valley area.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PREVALENS KEPELBAGAIAN GENETIK DI ANTARA KUMPULAN A DAN B VIRUS RESPIRASI SINSITIUM DIKALANGAN PESAKIT DI SEBUAH HOSPITAL PENGAJARAN DI KUALA LUMPUR.**

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Virus Respirasi Sinsitium (RSV) merupakan penyebab utama kepada jangkitan virus bahagian bawah sistem pernafasan di kalangan kanak-kanak. Jangkitan virus ini adalah bermusim dan kadar jangkitan meningkat pada musim sejuk dan hujan. Objektif utama projek ini adalah untuk menentukan prevalens RSV kumpulan A dan kumpulan B di Lebuh Klang dengan menggunakan kaedah tindakan rantai polymerase (PCR) sebagai satu kaedah yang mudah, cepat dan sensitif. Kaedah ini lebih sesuai dan sensitive berbanding kaedah imunofluresens untuk mengenalpasti RSV. Penyaringan dan pengenalpastian kumpulan RSV dilakukan dengan mengamplifikasikan gen-gen F dan G dari RSV. Primer yang spesifik dari gen F digunakan sebagai primer "antisense' untuk

penyaringan dan penentuan kumpulan RSV manakala gen G dari bahagian yang berlainan digunakan untuk tujuan yang sama.

Untuk mengetahui kepelbagaian RSV dalam penyelidikan ini, "Random Amplified Polymorphic DNA (RAPD) telah dilakukan pada kesemua 20 sampel RSV. Empat daripada lima belas primer yang digunakan telah menghasilkan banyak "bands" yang terang. Berdasarkan kepada dendogram yang dihasilkan daripada program perisian RAPDistance, sampel-sampel RSV berpisah dengan jelas kepada kumpulan masing-masing, Peratus persamaan di antara sampel- sampel RSV ini adalah dari 33% hingga 95% manakala jarak genetik Nei and Li's yang diperolehi adalah dari 0.0333 hingga 0.471.

Data yang telah diperolehi daripada penyelidikan ini hanya meliputi wilayah Lebuh Klang sahaja manakala, semua sampel yang dikumpulkan dari pesakit yang telah dimasukkan di wad kanak-kanak di Pusat Perubatan Universiti Malaya, Kuala Lumpur. Semua sampel RSV yang dipersolehi adalah dari bulan September 2002 hingga Mac 2004. Sampel-sampel ini telah diambil daripada pesakit-pesakit yang berumur satu bulan hingga satu tahun. Daripada dua puluh sampel yang diperolehi, , tiga belas sampel RSV ini telah diperolehi dari pesakit lelaki, dan yang lainnya telah diterima dari pesakit perempuan. Kadar jangkitan yang paling tinggi berlaku dikalangan bangsa Melayu diikuti dengan bangsa Cina dan India. Ujian "chi" kuasa dua telah dijalankan bagi memastikan data-data klinikal seperti umur, jantina dan bangsa mempengaruhi kadar jangkitan RSV. Dalam penyelidikan ini, umur, jantina dan bangsa pesakit yang dijangkiti RSV tidak

mempengaruhi kadar jangkitan. Tiada sebarang hubungan telah dikenalpasti diantara jangiltan RSV dengan data hip could be observed between the data demografik.

Daripada penyelidikan ini, RSV telah dikenapasti dengan berjayanya dalam 20 sampel mengunakan kaedah PCR. Gen-gen F dan G telah diamplifikasikan untuk mengenalpasti RSV dan membezakan RSV kepada kumpulan A dan kumpulan B. Didapati bahawa, jangkitan daripada kumpulan RSV A adalah lebih tinggi berbanding jangkitan daripada kumpulan RSV B dari bulan September 2002 hingga Mac 2004. Maka, kumpulan RSV A merupakan kumpulan yang prevalens di wilayah Lembah Klang.

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I certify that an Examination Committee met on 19th August 2005 to conduct the final examination of Vinomarlini Gunasagaran on her Master of Science thesis entitled "Prevalence of Genetic Diversity between Group A and Group B of Respiratory Syncytial Virus Isolated from Hospitalized Patients in a Teaching Hospital in Kuala Lumpur" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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The thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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17 NOV 2005

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Vinomarlini

**VINOMARLINI A/P GUNASAGARAN**

Date : 1/10/05.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| [T] | - test statistics |
| BRSV | - bovine respiratory syncytial virus |
| cDNA | - complementary DNA |
| $CO_2$ | - carbon dioxide |
| COPD | - chronic obstructive pulmonary disease |
| DNA | - deoxyribonucleic acid |
| dNTP | - deoxynucleoside triphosphate |
| EDTA | - ethylenediamine tetraacetatic acid |
| EMEM | - Eagle's minimal essential medium |
| F protein | - fusion protein F |
| G protein | - glycoprotein G |
| HHV | - Human Herpesvirus |
| HRSV | - human respiratory syncytial virus |
| kb | - kilo base pair |
| MAb | - monoclonal antibody |
| $MgCl_2$ | - magnesium chloride |
| mRNA | - messenger Ribonucleic Acid |
| NJTREE | - neighbour joining tree |
| OD | - optical density |

| | |
|---|---|
| PDA | - Patent Ductus Arteriosus |
| PCR | - Polymerase Chain Reaction |
| PIV | - Parainfluenza Virus |
| RAPD | - Random amplified polymorphic DNA |
| RNA | - Ribonucleic Acid |
| RSV | - respiratory syncytial virus |
| RT-PCR | - reverse transcribed- PCR |
| TAE | - Tris-Acetate-EDTA |
| UV | - ultra violet |
| Vero | - African Green Monkey Kidney Cell |
| mg | - milligram |
| ml | - milliliter |
| nm | - nanometer |
| µg | - microgram |
| µl | - microliter |

# CHAPTER 1

## INTRODUCTION

Respiratory Syncytial virus (RSV) is one of the most important causes of lower viral respiratory tract infections resulting in hospital admission among infants and early childhood worldwide. It is also considered as a serious problem in the elderly. The spectrum of infection ranges from mild upper respiratory tract disease to bronchiolitis and pneumonia. Most children recover from illness in 8 to 15 days (Collins *et al.*, 1996).

In the United States of America (USA), 50,000 to 80,000 infants each year are hospitalized and about 500 infants die each year because of the RSV infection. RSV infections cause symptoms like those of a common cold, a stuffy nose or runny nose, sore throat, wheezing and coughing, low- grade fever and earache. Babies may have additional symptoms that include listlessness, lack of appetite, irritability (fretfulness), disrupted sleep and a decreased interest in things going around them. Rarely, some babies may also have apnea, a condition where breathing stops for about 15 to 20 seconds. The majority of admissions to hospitals are due to bronchiolitis, the common lower respiratory tract manifestation of RSV infection (McNamara & Smyth, 2002).

The virus is a seasonal virus, with annual outbreaks occurring during the winter in temperate climates and during the rainy season in tropical climates. It is extremely

infectious and the predictability is similar among respiratory viruses. The virus causes syncytial masses when grown in culture and this is where the virus gets its name. Infection with RSV does not cause life long immunity. Children and adults may be infected year after year because of the presence of intratypic strains (McNamara & Smyth, 2002).

RSV has a single stranded, negative-sense RNA. It is classified in the genus *Pneumovirus* of the Paramyxoviridae family. It is a medium sized enveloped RNA virus and has a nucleocapsid morphology that is different than other paramyxoviruses that leads to the separate sub-family classification. The different nucleocapsid morphology is because the RSV has a N-bound RNA (which is referred as the nucleocapsid), which is considered one of the defining features of the *Paramyxoviridae* and is described as having a 'herringbone' appearance when imaged under a transmission electron microscope (TEM). Its viral genome is composed of 15,200 nucleotides and encodes 10 proteins. The virion is variable in shape and size (average diameter of between 120 and 300 nm), is unstable in the environment (surviving only a few hours on environmental surfaces), and is readily inactivated by soap and water and disinfectants (Feldman, *et al.*, 1999). The virus multiplies in the mucous membrane of the nose, throat and in the larynx. In infants, the virus may spread to the trachea, bronchi, bronchioles and alveoli. Fatal cases usually show extensive bronchiolitis and pneumonitis with scattered areas of atelectasis and emphysema resulting from bronchiolar obstruction (Chanock *et al.*, 1968).

RSV has 10 genes and the gene order is NS1, NS2, N, P, M, SH, G, F, M2 and L. The infection is initiated with the G protein binding to a host cell receptor, possibly a heparin-like glycosaminoglycan, followed by F protein mediated fusion of the viral and cell membrane, and penetration of the nucleocapsid complex into the cytoplasm (Feldman *et al.*, 1999).

RSV exists in two distinct subgroups, which is RSV group A and RSV group B. These two antigenic groups of RSV A and B have been identified based on monoclonal antibodies to the F gene (fusion protein) and the G gene (attachment protein). Both RSV subgroups are capable in infecting severe lower respiratory tract disease (Sullender, 2000). Even though, the clinical infection of both subgroups appears to be similar, infection with group A may produce disease with slightly greater severity (McConnochie *et al.*, 1990).

The molecular epidemiology of RSV in Malaysia is largely unknown. Currently, in Malaysia, there are no published data available on the epidemiology and the prevalence of RSV groups. The information of prevalence of the RSV groups is important to relate and to indicate the severity of the viral infection.

To conduct this molecular epidemiology study, Polymerase Chain Reaction (PCR) was used as a rapid detection and to identify the prevalence of RSV group A and B. The most common method for detecting RSV, which is used in hospitals, is by using direct immunofluorescence assay. This rapid technique gives a less sensitive result and could

not be used to subgroup the RSV. The sensitivity and specificity of the interpretable immunofluorescence assay direct stains in comparison with shell vial cultures were 85.9% and 87.1% respectively (Matthey, *et al.*, 1992). Thus, PCR is used as an alternative method because this method provides a sensitive tool for both detection and typing of RSV into groups. Identifying the prevalence of RSV groups will give extra advantage in preventing RSV infection. The information on prevalent of RSV subgroup would assist in the development of DNA vaccine.

**OBJECTIVE**

General Objective:

1. To establish baseline information on prevalence of RSV and the subgroups in the Klang Valley area.

The specific objectives of this research are:

1. To detect RSV by amplifying the F and G genes by PCR method.
2. To subtype RSV into subgroups A and B by seminested PCR.
3. To determine the diversity of the RSV strains based on the Random Amplified Polymorphic DNA (RAPD) technique.
4. To study the relationship between prevalence of RSV infection with the age, ethnicity and gender of patients with RSV infection.